– **Cloning of the HsRad52 gene.** The whole coding sequence of human Rad52 (HsRad52) was amplified by PCR from human thymus cDNA library. Sequences of the upstream and downstream primers are EG182: CGCGGATCCGATGTCT GGGACTGAGGAAGCAA (SEQ ID NO:1) and EG225: GTAGGATCCTGAGCCTCAGTTAAG ATGG (SEQ ID NO:2). Underlined sequences are homologous to the published 5' end and 3' end sequence of the HsRad52 gene (10, 12). For PCR, 0.5 μg of DNA was used in mixture containing each primer at 200 nM, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1 mM MgCl2, 0.01% gelatin, each of the four dNTP's at 200 μM and 2.5 units of AmpliTaq ploymerase. The reaction mixture was heated at 95oC for 3 min and used in a PCR consisting of 30 cycles of 94oC for 1 min, 60oC for 1 min, and 72oC for 1 min. The resulting DNA fragment was labeled with [-32P] dCTP by random priming (24) and used as a probe for isolation of a HsRad52 clone from human testis cDNA library in lambda Charon BS phage (25). DNA isolated from a hybridization-positive clone was digested by Not I and treated with T4 DNA ligase. The ligation mixture was used for transformation of E. coli SY204 . Ampicilin resistant transformants contained a plasmid, designated pEG970, which carries HsRad52 cDNA. The plasmid was used as a template for amplification of the coding region of HsRad52 gene by PCR reaction. The reaction was carried out by using Expand High Fidelity PCR Kit and primers EG182 and EG225. The resulting DNA fragment was inserted into expression vector pQE-31 in frame with 5' end sequence coding for a series of six histidine residues that function as a metal-binding domain in the translated fusion protein.–

On page 31, immediately preceding the claims, please insert the enclosed text entitled "SEQUENCE LISTING".

In The Claims

Please cancel claim 27.